range of shapes, and hence angles between the Fabs than after reconstitution of the freeze-dried mAb. It was hypothesized that the difference in angle distribution was due to loss of flexibility in the hinge region, which then impacted ability to bind target antigen.

A systematic study of the impact of excipients on conformational stability of two IgG1 monoclonal antibodies using a variety of biophysical techniques has been reported recently (Thakkar et al., 2012). The unfolding profile, aggregation behavior, and predenaturation transitions in response to solution conditions such as pH and temperature were different for the two IgG1 mAbs. The mAbs also showed different effective concentrations and effects from two stabilizing excipients as determined by differential scanning calorimetry (DSC), high-resolution ultrasonic spectroscopy, and fluorescent measurements. The main conclusion from these studies is that stabilizing excipients can have different effects on conformation and flexibility of mAbs of the same IgG1 class. The authors discuss in detail the differences in response to the solution condition and excipients between the two mAbs, and mention that the molecular origin of these differences still needs to be elucidated. These differences may be accounted for by differences in amino acid sequence that determine higher order structure. Alternatively the stressed conditions applied, such as extremes in pH or temperature, may result in covalent changes via chemical degradation routes, which in turn led to conformational changes. The latter was not investigated in these studies since no analysis of chemical alterations was done.

Usually conformational changes in proteins, especially when under storage at higher temperatures (>40 °C), result in protein aggregation so discussions that relate conformational changes to aggregation will be covered in the next section. In addition processing of mAbs to create freeze-dried formulations can result in conformational changes (Tian et al., 2007), and will be discussed in more detail in the chapter on formulation development.

Aggregation

Protein aggregation is one of the most common physical degradation routes, and has been summarized in several review articles and books (Mahler & Jiskoot 2012; Manning et al., 2010; Wang & Roberts, 2010). As previously discussed, aggregation can be induced by covalent chemical degradation routes. Although chemical degradation can result in aggregation, conformational changes can also lead to aggregation, very often as a result of exposure of interior hydrophobic amino acid residues.

As shown in Figure 3.1 these aggregates may be comprised of proteins mainly in their native conformation or denatured protein. The aggregates are considered "soluble aggregates" if there are no visible particulates in solution and the aggregates cannot be removed easily by filtration, which again may consist of either natively folded or denatured protein molecules. Issues have been raised regarding the lack of monitoring and quantification of subvisible aggregates that can exceed the solubility of the protein in solution resulting in particulates in the range of 0.1–10 mm. A big concern is that these particulates may be potentially immunogenic (Carpenter et al., 2009). However, it has been suggested that the link between the presence of these